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CARTILAGE DEGRADATION IN OA: A LIMITED ROLE FOR IL-1b

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Purpose: The main feature of osteoarthritis (OA) is degeneration and loss of articular cartilage. The cartilage matrix breakdown is due to both abnormal biomechanical stress and activation of catabolic processes involving metalloproteinases (MMPs). IL-1b is currently thought to have a prominent role in shifting the metabolic balance toward degradation. IL-1b is first synthesized as an inactive precursor, which needs a cleavage to turn into the secreted active form. This maturation process mainly occurs in a molecular scaffold called "inflammasome", where initiators (including NLRP3) and adaptor molecules (ASC) oligomerize and recruit pro-caspase-1. The complex formation induces caspase-1 activation, which in turn processes IL-1b precursor. Given the primary role of inflammasome in IL-1b maturation and the putative role of IL-1b in OA pathology, we aimed to clarify the role of both inflammasome and IL-1b cytokine in cartilage breakdown.

Methods: First, we investigated the expression of inflammasome components and we measured soluble IL-1b concentration by ELISA in the conditioned media obtained after 24 hours incubation with cartilage explants of 15 OA patients. Second, in primary mouse articular chondrocytes cultures, we used lipopolysaccharide (LPS, 0.01 to 1 μg/mL) to induce a pro-degradative phenotype, characterized by an increase in gene expression (real-time PCR) and in protein release of MMP-3 (ELISA), MMP-9 (zymography) and MMP-13 (Western-blot). We studied the effects of a deficiency in NLRP3 using chondrocytes from NLRP3−/− mice, of an inhibition of caspase-1 using Z-YVAD-FMK (10 μM) and of a blockade of IL-1b using an IL-1b receptor antagonist (IL-1RA, 100 ng/mL). At last, we triggered degradation in mouse cartilage explants by dynamic compression (0.5 Hz and 1 MPa of magnitude for 24 hours) to investigate the role of NLRP3 and IL-1b in load-induced cartilage degeneration.

Results: Despite the expression of the inflammasome components NLRP3, ASC and caspase-1 in OA chondrocytes, OA cartilage was not able to produce soluble active IL-1b itself. In mouse articular chondrocytes, LPS treatment dose-dependently increased MMP-3, MMP-9 and MMP-13 (538-fold induction in [MMP-3] in culture supernatant by LPS at 1 μg/mL). Surprisingly, this catabolic response was similar in NLRP3−/− chondrocytes (mean [MMP-3] ± SEM: 447±99 in WT versus 676±104 ng/mL in NLRP3−/− chondrocytes) and was unchanged with the caspase-1 inhibitor (mean [MMP-3] ± SEM: 1162±188 without versus 1229±223 ng/mL with Z-YVAD-FMK). These results demonstrate that the LPS-induced pro-degradative phenotype was inflammasome-independent. Being aware that other proteases can mediate IL-1b maturation, we used IL-1RA to block IL-1b activity. Once again, the LPS-induced catabolic response was unchanged (mean [MMP-3] ± SEM: 808±219 without versus 808±226 ng/mL with IL-1RA). Likewise, we characterized mouse cartilage explants degradation in response to dynamic compression, and we show again this load-induced catabolic response was NLRP3- and IL-1b-independent. Taken together, these results suggest that chondrocytes are able to acquire a pro-degradative phenotype without any contribution of IL-1b.

Conclusions: Our results challenge the view that IL-1b is a key mediator for cartilage degeneration in OA and may explain why previous trials with IL-1b inhibitors were all negative.

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ENHANCED APOPTOTIC AND REDUCED PROTECTIVE RESPONSE IN CHONDROCYTES FOLLOWING ENDOPLASMIC RETICULUM STRESS IN OSTEOARTHRITIC CARTILAGE

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Purpose: Endoplasmic reticulum (ER) stress has been shown to participate in many disease pathologies. Although recent reports have demonstrated that ER stress in chondrocytes is present in human osteoarthritis (OA), its role in the pathology of cartilage degeneration, such as chondrocyte apoptosis, remains unclear. The purpose of the present study was to investigate the association between ER stress and chondrocyte apoptosis in degenerative cartilage, and to clarify the involvement of ER stress in the pathology of OA.

Methods: Articular cartilage samples were obtained at total knee arthroplasty from the tibial plateaus of 11 patients suffering from knee OA. The histological severity of cartilage degeneration of each sample was evaluated by the Mankin scoring system. To evaluate ER stress in OA cartilage, the expression of phosphorylated PERK (pPERK), ubiquitin (Ub), GRP78, CHOP and phosphorylated JNK (pJNK) and the mRNA splicing of XBP1 (XBP1 splicing) in human OA cartilage by immunohistochemistry and RT-PCR, respectively. Chondrocyte apoptosis in OA cartilage was evaluated by immunohistochemistry for cleaved caspase-3 (C-CASP3). Additionally, human chondrocytes were treated with various concentrations of tunicamycin (0, 0.5, 1, 5, 10 μg/ml), an ER stress inducer, to assess the impact of ER stress on the mRNA expression of CHOP, XBP1 splicing and apoptosis, as determined by real-time PCR, RT-PCR and ELISA analysis, respectively.

Results: In human OA cartilage, the percentages of chondrocytes positive for pPERK, Ub and CHOP positively correlated with cartilage degeneration (Table 1) and the percentage of chondrocytes positive for C-CASP3 (Table 2). XBP1 splicing and GRP78 expression in severe OA cartilage containing the greatest number of C-CASP3-positive chondrocytes were similar to the levels in mild OA cartilage, however, XBP1 splicing was higher in moderate OA cartilage than in mild and severe OA cartilage (Table 1). Tunicamycin dose-dependently increased CHOP expression and apoptosis of cultured chondrocytes (Figure 1). Tunicamycin increased XBP1 mRNA splicing of chondrocytes, while the levels of the splicing in chondrocytes stimulated with 10 μg/ml concentration of tunicamycin were lower than the splicing levels stimulated by 1 μg/ml of tunicamycin (Figure 1).

Table 1. The relationship between ER stress and cartilage degeneration

	Mild (n = 5)	Moderate (n = 7)	Severe (n = 8)	Mankin score rs	Mankin score p-value
Immunohistochemistry					
pPERK(%)	23.0±10.4	37.0±5.0	49.0±7.0	0.86	0.001
Ub (%)	19.1±2.8	29.6±6.6	59.3±3.7	0.81	0.001
GRP78 (%)	16.2±2.9	21.2±1.9	24.2±2.8	0.51	0.028
CHOP (%)	8.2±5.2	15.9±13.7	35.4±9.2	0.74	0.002
pJNK (%)	8.6±7.5	32.7±14.9	48.6±13.8	0.81	0.001
mRNA analysis					
Spliced/unspliced XBP1	0.18±0.00	0.42±0.04	0.26±0.03	0.30	0.197

Table 2. The relationship between ER stress and chondrocyte apoptosis

	C-Casp3 rs	C-Casp3 p-value
Immunohistochemistry		
pPERK (%)	0.52	0.031
Ub (%)	0.49	0.039
GRP78 (%)	0.30	0.195
CHOP (%)	0.68	0.003
pJNK (%)	0.24	0.308
mRNA analysis		
Spliced/unspliced XBP1	0.34	0.147

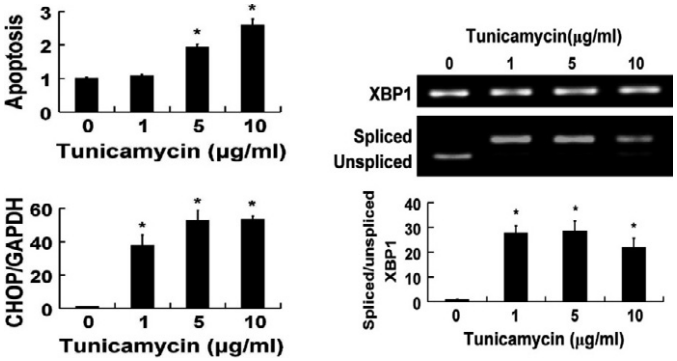

Fig. 1.

Conclusions: These results indicated that ER stress increased in cartilage during the progression of OA and that ER stress might contribute to chondrocyte apoptosis along with OA progression, which was closely